Reference: *Biol. Bull.*, **146**: 279–290. (April, 1974)

STUDIES ON THE MAINTENANCE OF ADULT SQUID (*LOLIGO PEALEI*). I. FACTORIAL SURVEY[1]

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Attempts to maintain cephalopods under laboratory conditions are usually concerned with a need to stabilize the supply of animals for various biological studies. Related to these basic investigations is the hope of learning more about the stocks of cephalopods in order to better exploit them for human food or, possibly, domesticate them in some form of mariculture. At the present time, it appears that the maintenance of cephalopods is replete with problems unique to each taxonomic group, as the life history differences might suggest. Maintenance success to date is apparently directly related to the benthic association of the group; thus octopuses in general are easier to maintain than cuttlefish, which in turn are easier to maintain than true squid. From practical considerations, species chosen for maintenance purposes should be readily available, small (in adult size), rapidly attain sexual maturation, have simple food preferences, and be tolerant of aquarium conditions.

The present study reports attempts to evaluate factors relating to the maintenance of the common Atlantic Coast squid, *Loligo pealei* (Lesueur, 1821). This animal is seasonally available (inshore), relatively large, reaches sexual maturity in one year (Summers, 1971), has only partially defined food requirements, and is notably intolerant of aquarium conditions (Summers and McMahon, 1970). It does, however, possess large axons which are the standard material for a considerable body of neurophysiological research and, as of the winter of 1969–1970, it has become the object of a sizable offshore fishery. A factorial approach was chosen in order to provide quantitative information which might lead to evenutal improvements in long term maintenance of *L. pealei.* Though we were aware of several rearing studies under way or completed at the beginning of this work, and had useful communications with Drs. J. M. Arnold, S. von Boletzky and E. T. LaRoe, our principal sources of information were summarized along with results of preliminary studies in an earlier paper (Summers and McMahon, 1970). Under rudimentary conditions, our experience had been that the survival of *L. pealei* was approximately 71% per day (a half-life of two days) over a period of one week. We had not succeeded in demonstrating differentials in survival related to the method of capture, water temperature, size, sex, or level of crowding.

MATERIALS AND METHODS

Source

Live squid (*L. pealei*) were taken with otter trawl nets on day trips within 20 km of Woods Hole, Massachusetts. Fishing locations and details of some of the trawl

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[1] This work was supported by NIH contract 69–2009 to Dr. Summers.

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equipment have been reported previously (Summers, 1968 and 1971; Summers and McMahon, 1970). Sixteen batches of squid were utilized. These resulted from captures ranging in date from May 11 to November 19, 1970. Ten batches were trawled by the R/V A. E. VERRILL, seven utilizing a 45–65 Long Island Sound balloon trawl (Summers and McMahon, 1970) and three utilizing a similar but smaller net designated as a 38–46 bar net by its maker, George W. Wilcox Company (Summers, McMahon and Ruppert, 1974). Six additional batches of squid resulted from trawling carried out by the commercial fishing vessel CAP'N BILL IV which utilized a #41 trawl net (see Summers, 1969). The first of these six batches was obtained by exclusive charter; the remainder were obtained through the Supply Department of the Marine Biological Laboratory which regularly employed the CAP'N BILL IV for summer squid collection.

Trawl contents were promptly emptied into seawater-filled tubs on the fishing vessels. Live squid were sorted into deck tanks (A. E. VERRILL) or a flooded fish hold (CAP'N BILL IV) and provided with running seawater until landed (a period of roughly one-half to four hours duration). Squid delivered by the Supply Department were subjected to repeated handling and sorting, often occupying as long as one hour, before being hand carried in tubs to the experimental tanks. Those obtained by direct charter of the CAP'N BILL IV and from the A. E. VERRILL were transferred into several 40-liter, polyethylene containers on the fishing vessel; these were filled to the top, fitted with tight lids (to prevent sloshing), provided with oxygen generating devices (O-Tabs), wheeled on hand carts, and finally hand carried to the experimental tanks. The entire procedure normally required about 20 minutes.

A total of 468 live squid were used in 16 experiments (341 males and 154 females). The disparate sex ratio resulted from sorting for large specimens. It was especially prominent among larger squid and among those obtained through the Supply Department. The numbers of squid used in the experiments varied from 16 to 48. Age composition estimated on the basis of population studies (Summers, 1971) suggested that 10% were young-of-the-year, 58% one year olds, and 32% two years olds. Young-of-the-year squid were predominant in the last four batches (capture dates: October 29 to November 19, 1970), one year olds were most common in the eight batches ranging from June 18 to October 15, 1970 and two year olds were numerous in the first 4 batches (May 11 to June 8, 1970).

Aquaria

Aquarium facilities for this study were built in a basement-level room approximately 6.7 m long by 6.1 m wide. Clerestory windows were provided on the northwest wall. These were fitted with venetian blinds and faced an adjacent brick building; thus, no direct sunlight shone into the room. The concrete walls and ceiling of the aquarium room were painted off-white and continuous, uniform illumination was provided by four tracks of fluorescent light fixtures located at ceiling level. Continuous air movement was maintained through the aquarium room and it was kept locked during experiments to avoid unnecessary disturbances. Convection heating/cooling units and nearby thermostatic controls held air temperature in the aquarium room to approximately 20–24° C.

Experimental aquaria consisted of four rectangular fiberglass tanks; two sizes each in two separate tank stands. A schematic presentation of the aquarium arrange-

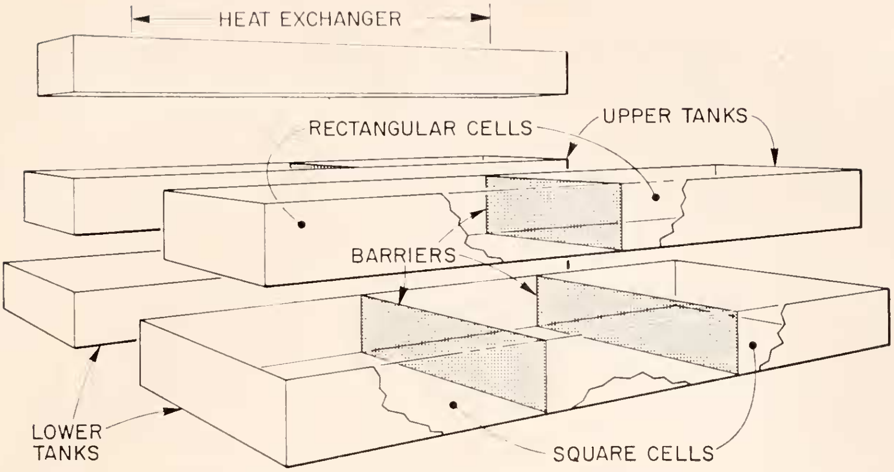

FIGURE 1. Schematic presentation of experimental aquaria in perspective as if viewed from the east. Windows in the aquarium room were located beyond the northwest ends of the tanks, at the right background. Portions of the northeast tank walls have been removed to show the position of barriers within the aquaria and the locations of square and rectangular experimental cells. Chilled seawater was available in the southwest tank stand (one upper and one lower tank) as a result of its passage through a reservoir containing a heat exchanger above these tanks.

ment is shown in Figure 1. All tanks had an inside length of 3.66 m. Lower tanks had an inside width of 1.37 m and upper tanks had an inside width of 0.92 m. Water depth was 0.31 m in all tanks, and the tank walls extended 0.05 m above water level. One tank stand was provided with an additional fiberglass reservoir of similar construction, 0.51 m wide, over the experimental tanks. All aquaria were opaque and coated with a smooth surfaced, buff-white gelcoat. They were made exceptionally rigid and partially insulated by the inclusion of $\frac{1}{2}$-inch (1.3 cm) end grain balsa core in the tank bottoms.

The reservoir held a galvanized steel heat exchanger consisting of ten vertical panels 2.72 m long by 0.30 m high. In conjunction with a $7\frac{1}{2}$ horsepower refrigeration compressor located outside the aquarium room, this heat exchanger functioned as an evaporator, allowing regulated cooling of the seawater entering one tank stand. The refrigeration unit could depress the temperature of a full flow of seawater about 10° C. Selected seawater temperatures were manually preset by adjusting the Freon pressure controls on the compressor. Sensitivity of the refrigeration unit was improved and (at low temperatures) formation of ice on the heat exchanger eliminated by reducing stratification of seawater in the reservoir. This was accomplished by running a stirring motor near the downsteam end of the heat exchanger and perforating the reservoir standpipe along its length with a number of small holes. Seawater temperatures in the chilled tank stand generally remained within a fractional degree Centigrade of preset temperatures.

Seawater was provided at one end of the upper tank and reservoir through polyvinylchloride (PVC) supply lines from the Marine Biological Laboratory seawater system; seawater passed the full length of each tank before cascading to the next lower level via "2 inch" PVC standpipes. Flow was held at approximately 2000 liters per hour in each tank stand. Higher flow rates produced considerable disturbance in the vicinity of the standpipes. This flow provided roughly two complete changes per hour in the upper (0.92 m wide) tanks and one and one-third changes per hour in the lower (1.37 m wide) tanks. Aeration occurred each time the seawater cascaded from one level to the next; mixing was aided by bubbling at the inlets and by continuous activity of the squid. Because the aquaria were new fabrication, they were cured at room temperature in air for several months, soaked in rapidly flowing seawater, and scrubbed periodically for a few weeks to dissipate any remaining solvents before the initiation of experiments.

All experimental tanks were fitted with lids made from corrugated fiberglass roofing material laid across the width of the tanks, overlapping where panels met and extending beyond the top tank edges. This material was selected for a spectral transparency similar to tabulated values (Svedrup, Johnson and Fleming, 1947) of clear coastal seawater. (In fact, except for a higher transparency in the red end of the visible spectrum, it closely mimicked typical values listed for one meter of coastal seawater.) The fiberglass material had the advantage of diffusing light and reducing sharp silhouettes. Lids mechanically prevented the escape of squid and very likely reduced the exchange of water with room air. Their presence also reduced possible tank fouling by airborne dust.

Movable barriers were constructed to divide the tanks into smaller compartments. These were made from rectangular frames of unpainted softwood lumber with knotless Nylon netting material (1.3 cm stretched measure) stapled over one side. Barriers were wedged inside the tanks with narrow splits of cedar shingles. In conjunction with the tank lids, the barriers effectively restricted squid to preselected segments of the experimental tanks.

Design

The experimental design called for the identification of factors significant in affecting the longevity of adult squid maintained under laboratory conditions. Because the supply of live squid was unpredictable, and earlier experience indicated that maintenance experiments might well extend beyond one week (Summers and McMahon, 1970), the design had to be both efficient and flexible. The basic design chosen was a 2^3 factorial experiment in which three factors were studied simultaneously, each at two different levels. Replications were to be provided within experiments in amounts depending upon the availability of live squid. Furthermore, experiments were to be repeated in suites of similar design in order to extract information on differences between batches of squid and maximize the evaluation of various factor combinations. Analysis was to be accomplished through an analysis of variance (ANOVA) of each individual experiment and of the pooled data from suites of experiments. Due to the seasonal nature of squid (Summers, 1969 and 1971) and ambient conditions in their native habitat (*e.g.*, seawater temperatures), it was anticipated that a number of observations could be made during the course of the experiments on factors which were independent of any particular experimental design. To this end, special care was taken to obtain the additional pertinent data.

It was recognized in advance that the two levels of each factor would have to be distinctive in order to properly evaluate the factor and that interactions beyond the first order would be poorly tested. Additionally, the 2^3 factorial design required a minimum of eight experimental "cells" necessitating the use of barriers, as described above and shown in Figure 1. Both upper tanks were divided in halves by single barriers at the midpoint, creating four "rectangular cells," 0.92 × 1.83 m by 0.31 m deep. Both lower tanks were divided in thirds with two barriers each, forming six "square cells," 1.37 × 1.22 m by 0.31 m deep. (Though not precisely "square," these cells were a reasonable approximation and crudely represented a regular polygon in contrast to the rectangular cells.) All cells had a plan area of approximately 1.68 m^2 and a capacity of approximately 520 liters. Because the four rectangular cells had only one netting wall each (the barrier), it was decided to use the four comparable square cells in the lower tanks for the experiments. Thus, the two center cells in the lower tanks (with two netting walls each) were not commited in the 2^3 factorial experimental design, and they were often used for the storage of spare squid. One odd experiment (the eleventh in consecutive order) was run in conjunction with the end of the previous experiment; it utilized the six square cells in a 2 × 3 factorial design.

It follows that any 2^3 factorial experiment utilizing these eight cells has cell shape as one factor, contrasting rectangular *vs.* square configurations at its two levels. When the refrigeration equipment was operated, another necessary factor was relative water temperature; contrasting ambient *vs.* a preset (chilled) water temperature. In operation, the chilled temperature was adjusted every few days to hold approximately a 5° differential between the two levels of this factor. The third imposed factor in the fifteen 2^3 factorial experiments was either feeding (levels: unfed *vs.* fed two live fish, *Fundulus* spp., per squid per day) or crowding (numbers ranging from one to eight squid per cell at paired levels determined by the available supply). The upper level of the feeding factor was arbitrary, though based on previous observations and was modified after the sixth experiment to prevent an indefinite accumulation of fish. The new level responded to demand by replenishing *Fundulus* daily, but not exceeding two per squid. Six experiments had the factors cell shape, feeding and crowding; six had the factors cell shape, feeding, and relative water temperature, and three had the factors cell shape, crowding, and relative water temperature. Where feeding was not a factor, all squid were fed as in the modified upper level given above. The 2 × 3 factorial experiment crossed relative water temperature with three square cells which were arbitrarily designated by their order relative to seawater flow.

During experiments, observations were made on an average of once every 9½ hours and no less than two hours nor more than one day separated consecutive observations. Typically, three observations were made during the working day (about four hours apart) and three over the weekend. Sporadic observations were made at other times during the course of these experiments. Elapsed time (to the nearest tenth of an hour) from the start of the experiment, date and time of day, barometric pressure, air temperature, ambient seawater temperature and chilled seawater temperature were logged at each observation; the last two were measured in the experimental tanks. Necessary operations were performed in or near the tanks and an accounting was taken of all squid at each observation, with as little disturbance as possible. Dead squid were removed, their dorsal mantle length, sex and sexual maturity were recorded with any comments on their condition. Survival time was considered to be the total elapsed time from being placed into the experimental tank to the observation of death; this was the principal statistic for analysis.

Operationally, tanks were drained, scrubbed (with bristle brushes and Nylon scouring pads but without chemicals), hosed down with fresh water and refilled just before another experiment began. Superfluous organic material of all forms, fresh squid eggs excepted, was removed at each observation. The experimental design was set and treatment combinations were randomized among cells before the experiments. (Because of physical restrictions, relative water temperature could not be crossed with tank stands and cell shape could not be crossed with upper and lower tanks; these factors were in fact nested.) On delivery, squid were immediately and impartially distributed among the experimental cells to the maximum number of whole replicates that the supply allowed. Refrigeration equipment was not started until the lids were in place and the squid had had a few minutes to adjust to the tanks. Chilling of the one tank stand produced measurable but transient temperature stratification which progressed first in the upper, than in the lower tank. It normally required 2–3 hours before stabilizing at the preset temperature. Feeding was begun on the morning following initiation of the experiment and was repeated daily at the first observation. Electrical services for the aquarium room, including the lighting, ventilating fan, and refrigeration system, were on circuitry which received emergency power in case of outages; these appliances were left on continuously and apparently functioned with no more than momentary failures through the 148 days required for the 16 experiments.

RESULTS

Mean survival of all squid was 87 hours (approximately $3\frac{2}{3}$ days) and individual experiments had mean survival times ranging from 58 to 142 hours. The maximum survival of an individual squid was 666 hours. There was mild statistical evidence for greater variability in survival times between batches of squid than within experiments. Analyses of variance demonstrated better than 95% confidence of significance for the factors cell shape, feeding and relative water temperature and the first order interaction of feeding and relative water temperature in at least one experiment each. Cell shape and relative water temperature produced better than 99% confidence of signficance in different experiments. Cell shape was the only factor demonstrated to have better than 95% confidence of significance in pooled data resulting from suites of similar experiments (it actually produced better than 99% confidence of significance).

A review of the data showed that rectangular cells promoted longer squid survival than square cells with a mean difference of nine hours overall. By construction, cell shapes were nested within tank heights, and these findings are confounded with the upper and lower tanks. No significance was demonstrated through the application of a chi-square test on pooled survival data for upstream *vs.* downstream pairs of cells in the same tank nor among cumulative data for the eight specific cells. The 2 × 3 factorial experiment did not show significance for the different square cells.

Seawater temperature ranged from 5.8–6.2° C to 20.1–22.6° C through the course of these experiments. Where relative water temperature was found significant (three out of a possible ten experiments), levels favoring survival differed.

The 2 × 3 factorial experiment indicated better than 99% confidence of a significance for relative water temperature, strongly favoring survival at the chilled level (11.1–11.7° C) contrasted with the ambient level (18.0–18.4° C) in a case where temperature differential was deliberately exaggerated. Attempts to correlate survival with absolute water temperatures were inconclusive. However, it was noted in the observations that squid kept at temperatures below 8° C behaved aberrantly and did not appear to feed readily (see Summers, 1969).

The crowding factor was studied at lower levels of two, three, and four squid per cell and higher levels twice those numbers in two experiments each (a total of six experiments). In addition, isolated individuals were contrasted with three, four and five squid per cell in one experiment each. In none of these nine cases was crowding demonstrated as a significant factor.

Feeding was utilized as a factor in twleve experiments and was found significant in only one. This, and the isolated occurrence of significance in one case of a first order interaction, can be attributed to chance.

There was mild statistical evidence (better than 90% confidence) for a difference between fishing vessels and their gear in the ultimate survival of squid. On an average, those trawled by the CAP'N BILL IV (and handled by the Laboratory Supply Department) survived ten hours less than ones trawled by the A. E. VERRILL.

Discussion

As can be expected, some aspects of the experiments had to be accommodated through modification of the experimental procedure. For example, the refrigeration unit was not completely installed and operational until the seventh experiment, which began on August 5, 1970. However, this corresponded with the occurrence of maximum ambient seawater temperatures and allowed study of the relative water temperature factor over the full range of ambient temperatures. The potential for ice formation in the reservoir was learned by experience and the stirring motor was not used until early November (during the fourteenth experiment). Though temperature control was maintained, it was found desirable to reduce the seawater flow rates during some coastal storms in order to minimize turbidity in the tanks.

During the first two experiments, four squid succeeded in jetting out of the tanks in spite of the lids. Because these "escapes" occurred in the first 2 days of both experiments, the squid were replaced by spare animals from the same batch which had been held at appropriate seawater temperatures in the lower tanks and were, at that stage, unfed. Various pieces of lumber were placed on top of the lids at this point, adding weight and reducing flexibility of the fiberglass materials; this prevented further squid escapes. Excepting the four escaped squid, all causes of mortality were accepted as appropriate experimental results. This included a few squid which were sufficiently weakened to be swept down the standpipes and were recovered on grating in the drain system.

Dead squid were frequently found in the corners of the cells and typically had damaged tail ends. This condition was manifest by abrasion of the skin and/or open wounds usually exposing the fractured tip of the pen. These animals showed progressive blunting of the tail end during maintenance, accompanied by an upward twisting at the tip. This condition is apparent in photographs of squid published by Voss and Sisson (1967). Some dead squid had been cannibalized, causing difficulty in determining their exact size and sometimes their sexual development. It was observed that moribund individuals were occasionally set upon by others, and this behavior was not correlated with the level of feeding. As reported previously (Summers and McMahon, 1970), cannibalism represented a limited, though real, source of nourishment for unfed squid.

Because computational facilities were available for the analysis of variance of 2^2 experimental designs having equal numbers of replicates in each data cell, the experimental results were regularly run three times, once for each pair of factors. Where crowding was one of the factors, some data was necessarily eliminated (by a random process) and the statistical results are conservative. The most powerful analysis of a factor pair was utilized in determining significance in any single body of data.

The presence of a galvanized heat exchanger in the seawater flowing to one tank stand was viewed as a potential factor in the survival of squid. Effects of zinc leaching from the heat exchanger were experimentally confounded with relative water temperature, which was not found to be frequently or consistently significant. In addition, the heat exchanger was not present in the first 6 experiments and a one-way analysis of variance for survival in the one tank stand, before and after its installation, was not significant. An analysis of dissolved zinc was performed by Dr. Derek Spencer of the Woods Hole Oceanographic Institution; it showed a ten-fold increase in the seawater flowing through the chilled tank stand compared with ambient seawater (44.0 ± 3.5 micrograms of zinc per liter compared with 4.5 ± 0.4 micrograms of zinc per liter). Reference was made to several published analyses of seawater, all of which gave zinc concentrations for typical coastal seawater intermediate to these two figures.

Failure to determine significance in the crowding factor was disappointing. Though it was observed that healthy undisturbed animals swam synchronously in groupings relatively smaller than the experimental cells, it had been anticipated that crowding would influence mechanical interaction with the tank walls and/or alter behavior patterns, thus altering survival. None was experimentally detectable. The isolation of individual squid in three experiments should have provided the strongest test for this factor, yet no effect was found. Nor was it possible to correlate the survival of squid with initial crowding using the pooled data from all the experiments. The isolation of one sex in a particular experimental cell by chance (23 cases) and by design (12 cases; isolated squid) did not produce survivals which were testably different from the experimental means, nor were survival differences between isolated males and isolated females found significant through the use of chi-square tests.

In our earlier work (Summers and McMahon, 1970), feeding had been avoided in order to study short-range effects. In the present study, feeding did not measurably improve survival. When fed, squid attacked and consumed live *Fundulus* in a deliberate fashion and defecated as evidence of digestion. It is possible that our imposed level of feeding was actually no better than starvation, thus preventing a statistical test of significance. We found it necessary to put *Fundulus* in the experimental cells one (or a few) at a time and observed that healthy squid would immediately seize them and withdraw in what appeared to be the peck order of that particular group. Proximity could alter this order of feeding, sometimes resulting in a dispute between squid. When several *Fundulus* were added simultaneously and/or were not taken when first presented, a single squid sometimes seized a number of them. In these cases, we were likely to overfeed in order to provide for the remaining squid. When moribund, squid often would not feed, hence our modification of the feeding level to prevent the accumulation of fish. The longest lived squid were in treatment combinations which included feeding. In view of these sparse facts, we hesitate to suggest that squid can be expected to survive indefinitely without feeding, but note that our results are anomalous.

Analyses of survival by age groups became simultaneously a study of survival by size because age groups were identified by size. Furthermore, different age groups appeared at different seasons, and one group (one year olds) had a seasonal change in sexual maturity (see Summers, 1971). For these reasons, Figure 2 shows survival by age group, size, sex, season and the significant factor, cell shape. As seen in part A of Figure 2, sexual dimorphism in dorsal mantle length becomes greater with age, as does the apparent effect of cell shape in the survival of squid. On overall mean, neither sex has a distinct survival advantage for any one age group. Insets in part B represent the range of survival times (shown in part A) and the period of principal occurrences for young-of-the-year and two year olds. Data for one year olds has been separated into two month periods in part B. Where seasonally mixed, it appears that older (larger) age groups have a survival advantage; especially late in the year when one year olds and young-of-the-year squid occur together. One year old males appear to survive longer than contemporary females during the first half of the year when they are both sexually mature and breeding.

We do not fully grasp the consistent significance of cell shape in the survival of squid. Were it not for the casual observation that squid oriented randomly relative to major cell dimensions, we might suggest that tank length determined this effect. Though differing in maximum length by nearly one-half meter, cell shapes were within one-quarter meter of the same diagonal measure and otherwise nearly equivalent. Order of a cell in the flow pattern did not appear to affect survival nor did the number of squid upstream from the experimental cell.

In a related study early in the season, a number of large squid (sexually mature males, 30–35 cm in dorsal mantle length) were driven the length of a long fiberglass tank to determine their swimming speed. the tank was 15.24 m long by 0. 92 m wide, filled to a depth of approximately 0.50 m and the timing course was the centrally located 12.19 m portion. When frightened by a brightly colored paddle, squid could be driven down the tank. Squid were usually clocked two or three times each before they tired (becoming slower in successive runs). Some evenutally attacked the paddle instead of completing the course. When fresh, these large squid swam at a sustained rate of approximately one meter per second, moving a mean one and one-third meters per jet pulse. This is consistent with calculated initial velocities of squid jumping free of the water, which were estimated at about 2 m/sec (*L. pealei,* Cole and Gilbert, 1970; *Loligo vulgaris,* Packard, 1969). Similar tests with smaller squid, including several females, showed that speed was roughly proportional to size, though jet pulse rate was the same for all sizes. If the maximum locomoter jet pulse of a squid is an "all or none" event (which the structure of its mantle nervous system suggests), then the "squid mean free path" appears to be no more than $1\frac{1}{3}$ m and likely about $\frac{2}{3}$ m for a typically-sized squid.

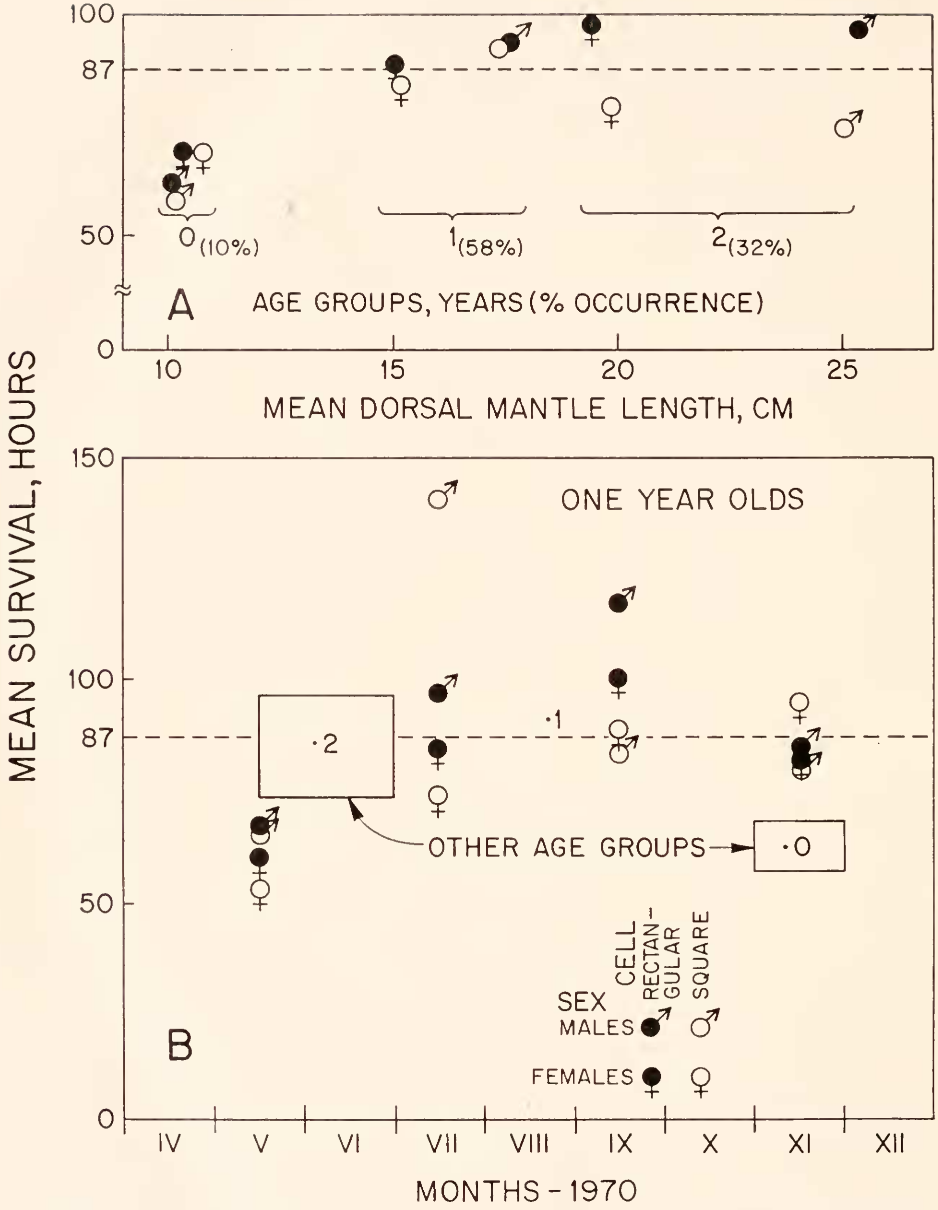

FIGURE 2. Mean survival of squid by size, age, sex, experimental cell shape and season. The overall mean, 87 hours, is indicated by a dashed line in both parts of the figure. Insets in part B represent the seasonal occurrence of a particular age group in the experiments and the range of survival times shown in part A, above. Data for the one year olds has been broken down into two month periods in part B. See text for discussion of the figure.

These figures are close to the dimensions of the experimental cells and may aid in evaluation of both absolute size and shape of maintenance tanks. (Note, a theoretical analysis of jet propulsion with reference to the squid *Loligo vulgaris* has been provided by Johnson, Soden and Trueman, 1972.)

Using the size data shown in Figure 2A, young-of-the-year squid, one year olds and two year olds had mean sizes (dorsal mantle lengths) of 10.5, 17.0 and 23.5 cm, respectively. Based on the foregoing empircal data, their respective sustained swimming velocities should be approximately 0.35, 0.57 and 0.78 m/sec. And, utilizing an approximate weight/length relationship (weight doubling for every 5 cm increase in dorsal mantle length; Summers, 1969), one can calculate kinetic energies of the different age groups at 15%, 100% and 470% relative to that of one year olds. With likely "squid mean free paths" of roughly 0.50, 0.75 and 1.00 m, respectively, the potential for collision damage obviously increases rapidly with size (and age). Given a hypothetical initial location central in any experimental cell and a random horizontal orientation, the chance that a young-of-the-year squid will strike the tank wall in one pulse is zero while that for a two year old is 100%. One year olds have probabilities of striking the tank wall of 31% in square cells and 53% in rectangular cells under these conditions. (Note, two-dimensional derivations of the ideal gas law also suggest more frequent collisions in oblong cells.)

Reference to Figure 2 shows that cell shape was especially important in survival of older age groups (larger squid), as expected, but favored rectangular cells in which collisions should have been more frequent. Netting walls in the barriers were 1½ times longer in the square cells, though their presence was not judged detrimental as tested by the 2 × 3 factorial experiment. Thus, we are bound to suggest that squid may orient in aquaria in such a way that they minimize collisions with the walls. Collison models built on these empirical data assume a horizontal plane of movement and provide logical mechanisms for the proportional survival model suggested in our earlier paper (Summers and McMahon, 1970). Present survival data, however, is not as well fit by the previous exponential model and the mean survival is longer.

Additional information on this subject and acknowledgments are given in Summers, McMahon and Ruppert (1974).

SUMMARY

1. This paper reports a factorial survey of conditions affecting the survival of the squid, *Loligo pealei,* in laboratory aquaria. Running seawater and continuous illumination were provided to various numbers of animals kept in 520 liter experimental "cells." A total of 468 squid were studied in 16 separate experiments run between May 11 and November 23, 1970.

2. Three imposed factors were studied simultaneously in any one experiment. These included feeding, crowding, relative water temperature and "cell" shape. The effects of uncontrolled factors such as ambient seawater temperature, age composition, size and sexual maturity were also evaluated.

3. Mean survival of all squid was 87 hours and maximum survival was 666 hours. Analyses of variance demonstrated better than 99% confidence of significance in the factor, "cell" shape (rectangular "cells" produced longer survival

than square ones). Mixtures of dissimilar age groups appeared to be detrimental in the survival of the younger group, and breeding animals (one- and two-year olds) did not live as long as some sexually immature individuals.

4. Collisions with the aquarium walls are discussed as likely causes of mortalities. Behavioral observations are used in evaluating the probabilities of these collisions.

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